Preparation and Characterization of fenugreek (*Trigonella foenum-graecum* L.) gum hydrogel and its application

प्रिपरेशन एंड कैरेक्टराइजेशन ऑफ फेनुग्रीक (*ट्राईगोनेला फीनम-ग्रेइकम* एल.) गम हैड्रोजेल एंड इट्ज ऍप्लिकेशन

A

SYNOPSIS SUBMITTED TO THE

DEPARTMENT OF FOOD SCIENCE & TECHNOLOGY,

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By

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Detailed synopsis for registration to Ph. D. program in Food Science & Technology

Proposed title of Ph.D. thesis: Preparation and characterization of fenugreek (*Trigonella foenum- graecum* L.) gum hydrogel and its application

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INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) belongs to legume family and finds its origin from South Eastern Europe and Western Asia, but now grown mainly in India and many parts of the world including Northern Africa and the United States (Altuntaş et al., 2005). Approximately 80% of the total world's production of fenugreek is contributed by India, and occupies an area of nearly 149,000 ha with total production of 202,000 metric tonnes (NHB, 2017-18). Fenugreek seeds contain a central hard and yellow embryo surrounded by a corneous and comparatively large, white and semi transparent layer of endosperm (Betty, 2008) which is further surrounded by a tenacious and dark brown husk. Fenugreek seeds contain high protein content (27.57%), soluble (30.6%) and insoluble (20.6%) dietary fiber, crude fat (6.71%) (Naidu et al., 2011) and minerals such as calcium, iron and β-carotene. The seeds are also rich in polysaccharides and galactomannan (Jiang et al., 2007). In India, boiling, pressure cooking, roasting or germination of fenugreek seeds is done to make the seeds soft, palatable and to remove its bitterness (Mathur and Choudhry, 2009). Fenugreek seeds are extensively used as spice in Indian cuisine for flavoring while its leaves, germinated seed and tender shoots are used as vegetables. The seeds are aromatic, pleasantly bitter and slightly sweet with anti-diabetic and hypocholesterolemic, galactogouge (Tiran, 2003; Zuppa et al., 2010) and carminative (Sauvare et al., 2000) properties. They are also reported to have remedial and nutritive properties and trigger digestion process (Srinivasan, 2006).

Fenugreek seeds and leaves also exhibit many medicinal properties (Srinivasan, 2006) and is one of the oldest medicinal plant being utilized in many Asian and African countries for its health benefits. It is found to exhibit many pharmacological effects such as hypoglycemia, hypocholesterolemia, anti-cancer, gastroprotective, anti-inflammatory, anti-anemic, and laxative

(Kumari and Sinha, 2012; Xue et al., 2007; Basch et al., 2003; Zia et al., 2001), and appetite stimulation attributes. These health benefits are known to be associated with dietary fiber and phytochemicals like phenols present in fenugreek (Mathur and Choudhry, 2009).

In fenugreek soluble fibers are identified as galactomannans (Jiang et al., 2007) with properties similar to soluble fiber of guar seeds and psyllium husk etc. Galactomannans are biopolymers consisted of linear core poly (1,4)-β-D-mannan backbone having varying degrees of D-galactosyl substituents attached by 1,6-glycosidic linkages (Im and Maliakel, 2008). Different galactomannans derived from various legumes usually differ in molecular weight, ratio of mannans to galactose and the mode and place of linkages of galactose moieties to the mannan backbone. For example, locust beans galactomanan have mannans to galactose ratio of 4:1, tara gum 3:1, guar seed 2:1 while highest amount of galactose observed in the fenugreek galactomannan, the ratio being 1:1. It is suggested that the mannose and galactose moieties are linked uniformly in fenugreek gum resulting in maximum hydration and solubility (Brummer and Cui, 2003; Im and Maliakel, 2008). This mannose and galactose ratio affects the physicochemical properties of galactomannans and inversely related to solubility of gum (Wu et al., 2009). Compared with guar gum, locust bean gum and tara gum; the highest galactose content was observed for fenugreek gum resulting in its highest water solubility (Wu et al., 1997). Galactomannans sterically stabilize the emulsions against flocculation and coalescence by getting adsorbed onto oil droplets. In addition to this, galactomannans are all found to exhibit some surface, interfacial, and emulsification activities (Shfeekh et al., 2008; Wu et al., 2009). Due to these properties, it is preferred over other natural hydrocolloids as an excellent ingredient for various food applications (Brummer et al., 2003). Thus, fenugreek gums with the highest galactose content (galactose to mannose ratio, G:M is 1:1) showed the maximum decrease of

cholesterol in both liver and blood plasma, as compared to guar gum and locust bean gum whose G:M are 1:2 and 1:4 respectively (Wu et al., 2009). As a hydrocolloid, the soluble fiber from fenugreek, referred to as fenugreek gum, provide texture, appeal, gelling, thickening, emulsifying, stabilizing and encapsulating properties. Thus dietary fiber, especially soluble fiber can find their way into nutrition and cereal bars, yogurts, dairy products and nutritional beverages. Plain powders of soluble fiber or total dietary fiber can be mixed with fruit juices, other spice mixes and seasonings. As direct supplements, it can formulate as tablets or capsules along with the other vitamins and nutrients. It may also be applied to milk shakes, soups, dressings, sweets and candies. It may also be used to fortify bakery flour for pizza, bread, pizza, bagel, muffins, cake mix, noodles, tortilla and flat bread, fried and baked corn chips etc. (Brummer and Cui, 2003; Im and Maliakel, 2008).

Hydrogel is hydrophilic polymer that has the ability to absorb water at physiological conditions such as pH, temperature and ionic strength because of the presence of a three-dimensional network structure (Mishra et al., 2018). The cross-links of the network may be represented by covalent bond, electrostatic, hydrophobic or dipole-dipole interactions while the hydrophilicity is attributed to the presence of hydrophilic groups, such as hydroxyl, carboxyl or amide groups along the polymer chain (Nilimanka et al., 2013). The polymeric hydrogel has two main features: cannot dissolve due to the covalent crosslink and the water uptakes far with hydrophilic linear polymers (Hoare et al., 2008). There are three integral parts of the hydrogels preparation: monomer, initiator and cross linker (Bajpai et al., 2008) prepared from materials such as gelatin, polysaccharides, cross-linked polyacrylamide polymers, polyelectrolyte complexes, and polymers or copolymers derived from methacrylate esters.

Swelling properties of hydrogels are most important for all polymer chains in hydrogels that are cross linked to each other either physically or chemically and thus, considered as one molecule regardless of its size. For this reason, there is no concept of molecular weight of hydrogels and therefore, sometimes called infinitely large molecules or super macromolecules. Hydrogels with acidic or basic functional groups respond to the fluctuations in the external environmental pH (Yang et al., 2012). Degree of ionization of the functional groups dictates its swelling profile and hence the volume changes. Mechanical properties of hydrogels are very important from the pharmaceutical and biomedical point of view. The evaluation of mechanical property is essential in various biomedical applications viz. ligament and tendon repair, wound dressing material, matrix for drug delivery, tissue engineering and as cartilage replacement material. The mechanical properties of hydrogels should be such that it can maintain its physical texture during the delivery of therapeutic moieties for the predetermined period of time (Yang et al., 2012). Recently, healable hydrogels with good fatigue resistant performance offer substantial benefits to maintain the integrity of network structure and mechanical level of bulk hydrogels, contributing to their long term use with stable functionality (Wei et al., 2015). The porosity of hydrogel allows drugs loading into the gel matrix, and then release, at a diffusion coefficient dependent rate of the gel network (Bajpai et al., 2008). Hydrogel are also used in, cosmetics, perfume, water beeds for plant, water adsorption nature of hydrogel is used in diaper that they hold water (Swarbrick, 2013).

The present study is proposed with the following objectives:

1. Extraction of fenugreek gum from fenugreek seeds
2. Preparation and characterization of fenugreek gum hydrogel(s) and its comparison with commercially available gums
3. Determination of thermal, morphological, and rheological properties of hydrogel(s)
4. Evaluation of prepared hydrogel(s) for various applications.

REVIEW OF LITERATURE

Fenugreek is an annual crop belonging to the Fabaceae family. Fenugreek seeds contain protein (27.57%), dietary fiber (soluble 30.6% and 20.6% insoluble), crude lipids (6.71%) and minerals including iron, calcium and β-carotene (Dhull and Sandhu, 2018). Kochar et al. (2006) reported that fenugreek seeds contain 11.8% moisture, 25.8% crude protein, 6.53% oil, 3.26% ash, and 6.28% crude fiber and 58.13% total carbohydrates on dry basis. El-Nasri and El-Tinay (2007) reported protein, fiber and fat content of fenugreek of be 28.4%, crude fiber content was 9.3%, and 7.1%. Pandey and Awasthi (2013) reported 45.5% of dietary fibers in raw fenugreek seeds. The fatty acid profile of fenugreeks is dominated by unsaturated acids, namely, oleic, linoleic, and linolenic acids accounting for 16.3%, 50% and 24.4%, respectively of the total fatty acids.

Mirhosseini and Amid (2012) reported that fenugreek gum is composed of α (1/4)-β-D-mannan backbone attached to single α-D-galactopyranosyl groups at the O-6 position of D-mannopyranosyl residues. The mannan backbone of FG is almost fully substituted with galactose side chains (Brummer et al., 2003). The hyper entanglement of unsubstituted mannan regions in FG becomes nearly impossible due to the presence of disaccharide repeating sequence in its primary structure. As reported by Brummer et al. (2003) the ratio of galactose to mannose in the extracted fenugreek galactomannans varied from 1.00:1.02 to 1.00:1.14.

Brummer et al. (2003) also investigated the effect of different extraction methods on the chemical composition of fenugreek gum. They reported that the cool water extraction yielded the gum with low protein content (2.36%) as compared to the solvent extraction using boiling hexane. The protein level was further decreased to 0.57% using the pronase hydrolysis. Huang et

al. (2001) reported that the extracted FG contained 0.8% residual protein, thereby prohibiting the function of surface activity to the hydrophilic gum alone. Huang et al. (2001) evaluated 11 commercial gum along with fenugreek gum and found that FG had the highest stabilizing capacity amongst other 11 commercial gums and 5 laboratory synthesized gums (i.e. Gellan, carrageenan, pectin, methylcellulose, gum Arabic, microcrystalline cellulose, locust bean gum, guar, xanthan, mustard, oat gums, flaxseed and oat gums).

Ahmad et al. (2019) reported potential uses of natural gum polysaccharides in numerous aspects of biotechnology, food, environment and medicine industries. They also reported that the natural gums represent a great extent unexplored source of significant natural products and contribute to assorted variety as a critical segment of worldwide foodstuff, paper, remedial, texture and other markets (Ahmad et al., (2019). They classified different groups of natural gums hydrocolloids relying upon their source, functional properties, structural properties and applications namely bacterial polysaccharides (gellan gum, dextran and xanthan), botanicals (guar gum, locust bean gum and pectin), tree gums (arabic, karaya, tragacanth, ghatti, and kondagogu) and seaweed polysaccharides (alginate, carrageenan, and agar).

Hydrogels is a kind of polymeric materials with a three-dimensional structure maintained by covalent or non-covalent intraction between macromolecules (Kopecek, 2007). The hydrogel properties mainly depend on the cross-linking degree, on the chemical composition of the polymeric chains, and on the interaction between the network and the surrounding liquids (Ciolacu and Cazacu, 2011). Due to the cross-linking between the network chains, they have the ability to retain water in their structure without getting dissolved (Ahmed, 2019).

Wang et al. (2019) investigated that hydrogels have excellent mechanical properties, biocompatibility, and versatility (i.e. self-healing ability, viscosity and toughness). Due to the presence of gelatin and gum arabic aldehyde, hydrogels exhibited excellent biocompatibility thus, could be used as a potential drug carrier.

Zhang et al. (2018) investigated hybrid hydrogels by combining the hyperbranched polymers with xanthan gum. They reported that hybrid hydrogels have self-healable, porous structure and release controlled characteristics. Hyperbranched polymers have low viscosity at very high concentrations. Thus, it does not easy to prepare hydrogels from them directly. Hybrid hydrogels were synthesized with mushroom hyperbranched polysaccharide and XG by esterification and crosslinking reaction using STMP (sodium trimetaphosphate) as crosslinker.

Mishra et al. (2018) reported that hydrogels are successfully used in biomedical field in adverse environmental conditions like low ph and high temperature in human metabolism. A large no. of hydrogel materials has been investigated for controlled drug release for drug formulation purpose. This bio-compatible nature of hydrogels makes them a promising candidate for future applications as well as for the development of next generation of materials for biomedical applications also environment field. They also concluded that a major weakness of all these hydrogels is the need for optimization of their response time, which is either to slow or too fast.

Mohammadi et al. (2019) reported that the Gum Tragacanth, as a suitable material for immobilizing TiO_2 nanoparticles to remove cationic dye from wastewater. The nanohydrogel were prepared by dispersing TiO_2 nano-particles in Gum Tragacanth solution and the subsequent addition of glutaraldehyde as the crosslinking agent.

Shafagh et al. (2018) evaluated a pH-sensitive and antibacterial drug releasing systems through a completely green route. They reported that the gelatin natural biopolymers were cross-linked with citric acid in the presence of Ag nano-particles (NPs) also found that these hydrogels have pH-dependent swelling and drug release behavior with higher drug release at pH 7.4 compared to pH 1.2. Also, as an antibacterial effect against the E. coli and S. aureus microorganisms were achieved by incorporation of Ag NPs into hydrogels.

Kestwal et al. (2015) investigated a fenugreek hydrogel agarose composite entrapped gold nanoparticles for acetycholinesterase based biosensor was fabricated for detection of various pesticides. In this novel bio-composite matrix (including fenugreek hydrogel and agarose) were used to immobilize the enzyme and nano-particles. The enzyme stability was markedly improved due to immobilization.

Lim et al. (2014) utilized hyaluronic acid and polyethylene glycol based hydrogel nanoparticles as effective carriers in transdermal delivery systems. They reported that hydrogels enhanced drug delivery efficiency in the skin with the help of nanoparticle. Hydrogels exhibited to improved water absorbing and water retention capability, swelling rate, pH-resistant property and reusability prepared by (Wang et al., 2014). The properties of carboxymethyl and hexanoyl substituted chitosan based hydrogels were pH sensitivity. The pH sensitivity of the hydrogels was more pronounced in carboxymethyl substituted chitosan as compared to hexanoyl group substituted chitosan due to the altered state of water absorption and caused by inhibiting the intermolecular hydrogen bonding (Liu and Lin, 2014).

Ozcelik et al. (2014) investigated the ultrathin chitosan-poly (ethylene glycol) based hydrogel films for corneal tissue engineering application. They observed that through variation of the poly (ethylene glycol) content it was possible to tailor the hydrogels to have tensile strains

and ultimate stresses identical to or greater than those of human corneal tissue; while, retaining similar tensile moduli.

Xiao et al. (2014) evaluated the properties of the hydrogel films prepared from chitosan and hydroxypropyl guar gum; and determined improvement in tensile strength and elongation at break with increased concentration of hydroxypropyl guar gum in chitosan. Pal et al. (2019) reported that the attributes of anticancer therapeutic embedded in programmable gum ghatti hydrogel matrices. Hydrogel has been programmed for adjusting the product composition (monomer-acrylamide and crosslinker- N, N Methylenebisacrylamide (MBAm)) to control mesh size during copolymerization of gum through microwave based technique. Rao et al. (2018) investigated a self-healing alginate/guar gum hydrogel, using polydopamine-type microcapsules from mesoporous silica nanoparticles. They reported that the reversible non-covalent bonds were responsible for the energy dissipation and network reformation. The tensile strength and healing efficiency of self-healing hydrogels can reach about 7.0 MPa and 94.5%, respectively.

Dai et al. (2019) investigated a method of guar gum (GG)-based self assembling hydrogel to impart excellent oil/water separating capability so that it can be applied for oily waste water treatment. The method were demonstrated by coating the guar gum (GG)-based self-assembling hydrogel on to a cotton fabric, They also investigated that GG hydrogel-coated cotton fabric exhibits excellent oil repellence. The oil/water separation efficiency is 97.5%, with high water flux (1467 Lm−2 h−1). The alkaline and salty conditions have no negative effect on the oil/ water separation efficiency.

Pande (2017) reported that the hydrogels were widely use in cosmetics, perfume delivery, water beeds for plants, water adsorption nature of hydrogels used for diaper that they

hold water, creams, perfume. They also use in environment for waste water treatment that can hold micro-organism in their matrix. Biosensors can be prepared by hydrogels, act as supports for immobilization of enzymes. The hydrogel polycarbamoylsulphonate is used for immobilization of the D-fructose dehydrogenase enzyme (Swarbrick, 2013). Hydrogels were used in biomedical applications such as, in immunotherapy, vaccine, plastic surgery, wound healing, electrophoresis, proteomic, tissue engineering (bone regeneration, cardiac and dental), drug delivery, wound dressing and so on.

Significance of the study:

Gums have some favorable features, such as great structural versatility, high water-affinity, and easy procedures, which facilitates the general applications of polysaccharide-based materials in food, packaging and pharmaceutical field. Fenugreek gums are rich source of polysaccharide galactomannan. It consist highest amount of galactose, the ratio of mannose to galactose being 1:1 compared with other gums (guar gum 2:1, tara gum 3:1 etc.); resulting in maximum hydration, solubility and swellability. Fenugreek gum also exhibited the highest stabilizing properties as compared to other gums. Due to these properties, it is preferred over other natural hydrocolloids as an excellent ingredient for various food applications. So, the properties of fenugreek gum can be utilized in preparation of hydrogels which may be used to develop new process and consequently new food products. Hydrogel microspheres may be useful for a variety of applications within the personal care, food, and pharmaceutical industries. In addition, they may also be used to protect, encapsulate, and release bioactive agents, such as pharmaceuticals, cosmetics, or nutraceuticals. The potential of using hydrogels formed from gums, in replacing fat and starch granules, may be promising for the development of healthy reduced-calorie foods. Thus soluble dietary fiber, in the form of hydrogel can find their way into nutrition and cereal bars, yogurts, dairy products and nutritional beverages. Hence, the present research aimed to prepare and characterize fenugreek gum hydrogel; can provide valuable information which can be useful for health and food industries.

MATERIALS AND METHODS

Materials: Indian fenugreek cultivars will be procured from suitable sources.

Methods:

Fenugreek gum: The gums will be extracted from fenugreek seeds using standard protocols.
Preparation of hydrogel(s): The hydrogel(s) of fenugreek gum will be prepared by standard method.
Significant properties of hydrogel(s): Various properties (Swelling, water vapour transmission rate, gel fraction, moisture content, bonding strength of hydrogel(s) etc.) will be determined.

Thermal, morphological and rheological properties: Thermal, morphological and rheological properties of hydrogel(s) will be determined by using Differential Scanning Calorimeter, Scanning Electron Microscopy and rheometer etc.

Applications of hydrogel(s): Applications of hydrogel(s) in food will be explored.

Statistical Analysis: Data obtained through experimentation will be statistically analyzed using suitable software.

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